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TITLE: The Fanconi Anemia BRCA Pathway as a Predictor of Benefit from Bevacizumab in a Large Phase III Clinical Trial in Ovarian Cancer

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14. ABSTRACT

We have completed DNA sequencing using BROCA-HR assay developed by our group for 65 DNA repair genes usin blood DNA from two clinical trials in advanced ovarian cancer, GOG 218, N=773 and GOG 262, N=573. 278 (14.7%) had mutations in *BRCA1* (182) and *BRCA2* (96). 109 (5.8%) had 112 mutations in the following genes: *BRIP* (27), *CHEK2* (13), *RAD51D* (11), *PALB2* (11), *ATM* (11), *RAD51C* (10), *NBN* (9), *TP53* (6), *BARD1* (4), *MSH6* (4), *FAM175A* (3), *PMS2* (2), and *MLH1* (1). Consistent with their role as ovarian cancer susceptibility genes, *BRIP1*, *RAD51C*, and *RAD51D* were significantly more frequently mutated in OC than in the ESP (all P<0.001). *PALB2* and *BARD1*, which are not proven ovarian cancer genes, were also significantly more frequently mutated in OC (*PALB2*: OR of 11.0 , 95% CI [2.4 – 50], p=0.0003; BARD1: OR 31, 95% CI [1.7 – 577], p=0.02. *ATM*, *NBN*, *CHEK2*, and *FAM175A* mutations were not significantly more common in OC. Overall, we determined that at least 11 genes contribute to ovarian cancer and explain 20% of cases.

15. SUBJECT TERMS

Ovarian, angiogenesis, clinical trial, homologous recombination, mutation, BRCA1, BRCA2, PALB2, BARD1, RAD51C, RAD51D, BRIP1, outcomes, histology

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INTRODUCTION:

The Gynecologic Oncology Group (GOG) trial 218 was a randomized phase III trial in primary stage III and IV ovarian carcinoma, which found a statistically significant improvement in progression free survival (PFS) for combination chemotherapy with extended bevacizumab, an antibody to the VEGF receptor that inhibits angiogenesis. However, this relatively small increase in PFS came at high financial cost and increased toxicity. Identifying biomarkers that predict increased response to bevacizumab is essential to allow more individualized and cost effective patient care. Inherited mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) occur in 13-18% of all ovarian carcinomas. *BRCA1*/2 are involved in the Fanconi anemia (FA) DNA repair pathway and *BRCA2* is a FA gene. Women with *BRCA1*/2 mutations are known to have an improved overall survival compared to women with sporadic ovarian carcinoma. The FA-BRCA pathway controls DNA repair via homologous recombination (HR). Recently, our group and others have more broadly implicated the FA-BRCA pathway in the etiology of hereditary ovarian carcinoma, identifying a number of new ovarian cancer susceptibility genes including *BARD1*, *BRIP1*, *RAD51C*, and *RAD51D*. Our hypothesis was that women that are wildtype for germline mutations in the FA-BRCA pathway would demonstrate greater benefit from the addition of extended bevacizumab to standard induction chemotherapy for advanced ovarian carcinoma.

KEYWORDS

Ovarian cancer, angiogenesis, homologous recombination, mutation, BRCA1, BRCA2, PALB2, BARD1, RAD51C, RAD51D, BRIP1, outcomes, histology

OVERALL PROJECT SUMMARY

To date, we have achieved all projected milestones on time.

Current objectives: Our primary objective is to deeply sequence a large number of genes in the FA-BRCA pathway in germline DNA from GOG218 and correlate the presence of deleterious mutations with progression-free survival according to treatment arm. Secondarily we are assessing the contribution of novel FA-BRCA genes to hereditary ovarian carcinoma including those of various histological sub-types and correlating the presence of FA-BRCA mutations with overall survival and treatment toxicity.

Summary of results, progress and accomplishments

Major activities**:** We have completed DNA sequencing using the BROCA-HR assay developed by our group for 65 DNA repair genes using blood DNA from GOG 218, N=773 and GOG 262, N=573. We initially planned to sequence 1200 cases from GOG 218, but germline DNA was not available from as many cases as projected. Therefore, to complete our goal to sequence germline DNA from 1200 unselected women with advanced ovarian cancer, we supplemented our GOG-218 series with 573 blood DNAs from another phase III GOG trial, GOG262. In order to achieve our stated goal for the GOG-218 treatment analyses, in year 2 we will supplement the blood DNAs already sequenced with another 500 tumor DNAs. These additional samples will allow us to evaluate both germline and somatic mutations in our target genes.

Germline mutation rates excluding copy number variations (CNVs) were compared to European American (EA) population rates from the NHLBI GO Exome Sequencing Project (ESP). ESP insertions and deletions were hand curated. Only clearly damaging mutations were included. We combined our germline mutation data from GOG218 and GOG262 with an additional 543 unselected ovarian cancer cases from the University of Washington. In total, 381/1889 (20.2%) women had 390 germline mutations in cancer-associated genes. 8/381 (2.1%) had more than one

mutation. 278 (14.7%) had mutations in *BRCA1* (182) and *BRCA2* (96). 109 (5.8%) had 112 mutations in the following genes: *BRIP1* (27), *CHEK2* (13), *RAD51D* (11), *PALB2* (11), *ATM* (11), *RAD51C* (10), *NBN* (9), *TP53* (6), *BARD1* (4), *MSH6* (4), *FAM175A* (3), *PMS2* (2), and *MLH1* (1). Consistent with their role as ovarian cancer susceptibility genes, *BRIP1, RAD51C*, and *RAD51D* were significantly more frequently mutated in OC than in the ESP (all P<0.001). *PALB2* and *BARD1*, which were not proven ovarian cancer genes, were also significantly more frequently mutated in OC (*PALB2*: OR of 11.0 , 95% CI [2.4 – 50], p=0.0003; BARD1: OR 31, 95% CI [1.7 – 577], p=0.02. *ATM, NBN, CHEK2,* and *FAM175A* mutations were not significantly more common in OC.

In GOG 218, germline *BRCA2* mutations were associated with significantly improved progression-free and overall survival (p=0.009 and p=0.0005, respectively). *BRCA2* carriers had more grade 4 neutropenia (p=0.04). Histologic subtype, race, ethnicity, and primary site did not predict mutation status. In summary, we found that mutations in *PALB2*, *BARD1*, *BRIP1*, *RAD51C*, and *RAD51D* were more frequent in OC than in the ESP EA population.

Discussion: We have completed the sequencing of a large number of germline DNAs from two phase III ovarian cancer trials and identified two new ovarian cancer susceptibility genes, BARD1 and PALB2. This brings the total of known ovarian cancer susceptibility genes to 11 and suggests that 20% of ovarian cancer is hereditary. Histology is not a significant predictor; therefore, all women with ovarian cancer should be offered genetic testing without regard to age, race, or histology.

KEY RESEARCH ACCOMPLISHMENTS

- Identified 11 hereditary ovarian cancer genes significantly more frequently mutated in women with ovarian cancer than in cancer free controls, including 2 new ovarian cancer susceptibility genes PALB2 and BARD1

CONCLUSIONS

These data imply that up to 20% of ovarian cancer is preventable with widespread uptake of genetic testing and timely surgical prevention for high risk women. If achieved, more comprehensive prevention of hereditary ovarian cancer would have a significant impact on overall cancer mortality. Women in the military as well as civilian women should be assessed for familial cancer risk and referred for genetic testing when appropriate. In the next time period we will complete the sequencing of the GOG218 samples and test our primary hypothesis to determine if mutation status is a predictor of benefit from extended bevacizumab therapy.

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